J. Parasitol., 90(2), 2004, pp. 255–259

EXPERIMENTAL *TOXOPLASMA GONDII* INFECTION IN GREY SEALS (*HALICHOERUS GRYPUS*)

A. A. Gajadhar, L. Measures*, L. B. Forbes, C. Kapel†, and J. P. Dubey‡
Centre for Animal Parasitology, Saskatoon Laboratory, Canadian Food Inspection Agency, 116 Veterinary Road, Saskatoon, Saskatchewan S7N 2R3, Canada. *e-mail: agajadhar@inspection.gc.ca*

ABSTRACT: Laboratory-reared animals were used to assess the susceptibility of seals (*Halichoerus grypus*) to *Toxoplasma gondii* infection. Four seals were each orally inoculated with 100 or 10,000 oocysts of *T. gondii* (VEG strain), and another 4 seals served as negative controls. Occasionally, mild behavioral changes were observed in all inoculated seals but not in control animals. A modified agglutination test revealed the presence of antibodies to *T. gondii* in sera collected from inoculated seals and mice inoculated as controls. No evidence of the parasite was found on an extensive histological examination of seal tissues, and immunohistochemical staining of tissue sections from inoculated seals revealed a single tissue cyst in only 1 seal. Control mice inoculated with 10 oocysts from the same inoculum given to seals became serologically and histologically positive for *T. gondii*. Cats that were fed brain or muscle tissue collected from inoculated seals passed *T. gondii* oocysts in feces. This study demonstrates that *T. gondii* oocysts can establish viable infection in seals and supports the hypothesis that toxoplasmosis in marine mammals can be acquired from oocysts in surface water runoff and sewer discharge.

Toxoplasma gondii is an intracellular protozoan parasite capable of infecting virtually any species of homeotherms, including humans. Ingestion of infected tissues or sporulated oocysts and transplacental transmission are the primary means of acquiring *T. gondii*. Sexual reproduction of the parasite occurs in felids, which are the only known definitive hosts. Oocysts passed in the feces of infected cats require a few days of adequate temperature and moisture to sporulate and become infective to birds and mammals, which serve as intermediate hosts. Oocysts are disseminated through fecal contamination, and water-borne transmission has been implicated in outbreaks of human toxoplasmosis (Aramini et al., 1999; Tenter et al., 2000). Also, there is evidence to suggest that invertebrate transport hosts may play a role in the transmission of oocysts (Wallace, 1972).

Over the past few decades, there have been increasing numbers of reports on *T. gondii* infection in a variety of marine mammals, including whales, dolphins, seals, sea lions, sea otters, and manatees (Migaki et al., 1977; Buergelt and Bonde, 1983; Cruickshank et al., 1990; Mikaelian et al., 2000; Lambourn et al., 2001). Although previous studies described histological findings, recent reports provide serological, immunohistochemical, polymerase chain reaction, bioassay, and in vitro culture evidence of the parasite in marine mammals (Lindsay, Thomas et al., 2001; Miller et al., 2001). However, direct evidence linking a terrestrial source of *T. gondii* has not been demonstrated, and transmission dynamics of the infection among marine hosts are unknown. No experimental work with *T. gondii* in marine mammals has been reported.

It has been suggested that toxoplasmosis in marine mammals is often associated with morbillivirus or immunosuppression (Mikaelian et al., 2000) and that anthropogenic environmental changes may be contributing to the emergence of *T. gondii* infections in marine mammals (Miller et al., 2002). Although the disease and its economic impact on terrestrial animals have been described, the effect of the parasite on the health and behavior of marine mammals is unknown. Nevertheless, protozoan encephalitis has been reported as the cause of disease and death in some marine mammals (Mikaelian et al., 2000; Lindsay et al., 2001). In this study, we used oocysts of a moderately virulent *T. gondii* terrestrial strain to establish experimental infections in gray seals and then completed the life cycle in cats. We also attempted to determine the clinical effects of experimental infection on behavior of seals.

MATERIALS AND METHODS

Parasites

Oocysts of the VEG strain of *T. gondii* were obtained from cats as described previously (Dubey et al., 1996). The isolate used is a type III strain and is mildly virulent to mice, depending on the stage inoculated.

Animals

Eight female seal pups (*Halichoerus grypus*), born and weaned naturally after 2-wk lactation on ice in the Gulf of St. Lawrence, were captured and transported to the Maurice Lamontagne Institute, Mont-Joli, Quebec, Canada. Seals were tagged for identification and weighed and bled for serum collection on a weekly basis. Seals were housed and maintained in 2, 13,000-L tanks and fed wild-caught herring that were stored at −20 C for several weeks. Seals were given daily supplements of Sea-Tabs™ consisting of vitamins, salts, and minerals (Pacific Research Laboratories Inc., El Cajon, California). At inoculation, seals were 8- to 12 wk old and weighed at least 50–56 kg. *Toxoplasma gondii*–free outbred CDI mice, 10–12 wk of age, were obtained from a commercial supplier and served as inoculation and virulence controls for the *T. gondii* isolate used in this study. Five neutered domestic shorthair cats, born, housed, and raised in the laboratory under specific pathogen-free conditions for *T. gondii* were 1–1.5 yr of age at the start of the experiment. All seals and mice were observed 1–4 times daily for clinical signs and any change in behavioral patterns.

Experimental design

The infectivity and virulence of the *T. gondii* VEG strain isolate used in this study were assessed by a titration infection experiment in mice. Mice in 8 groups of 3 were fed oocysts ranging in number from 1×10^4 to 4×10^4.

Four seals housed in a single tank were inoculated orally with *T. gondii* oocysts suspended in water in gelatin capsules. One hundred oocysts (low dose) were given to each of the 2 seals, and the other 2 seals received 10,000 oocysts each (high dose) (Table I). Four control seals were kept together under similar conditions and used in a *Trichinella spiralis* infection study. Blood samples from each seal were collected weekly from the extradural intravertebral vein. One seal each

Received 16 May 2003; revised 17 July 2003; accepted 22 July 2003.
* Fisheries and Oceans Canada, Maurice-Lamontagne Institute, Mont-Joli, Quebec, Canada.
† Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, DK-1870 Fredericksberg C., Denmark.
‡ United States Department of Agriculture, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville, Maryland 20705-2350.

TABLE I. Experimental design of *Toxoplasma gondii* transmission study showing the dose (number of oocysts), the time of necropsy (week PI), and serology results for inoculated and control seals, mice, and cats.

Animal no.	Dose (oocysts)	Necropsy (wk)	PI serology 1:40	PI serology 1:4,000	Week sampled*
Seal 101	100	10	+†	+	3
			+	+	8
			+	+	10
Seal 102	100	5	+	+	3
			+	+	5
Seal 103	10,000	10	+	+	3
			+	+	8
			+	+	10
Seal 104	10,000	5	+	+	3
			+	+	5
Seal 105	0	5	P‡	−§	3
			P	P	5
			P	P	10
Seal 106	0	5	−	−	3
			−	−	5
			−	−	10
Seal 107	0	10	−	−	3
			−	−	5
Seal 108	0	10	P	−	3
			P	P	5
Mice (×6)	10	5	+	+	5
Mice (×6)	10	10	+	+	10
Mice (×6)	0	10	−	−	10
Cat 7	Seal 102		+	+	23
Cat 11	Seal 104		+	+	23
Cat 15	Seal 101		+	+	19
Cat 21	Seal 103		+	+	19
Cat (control)	None		−	−	19

* Week of blood collection relative to week of inoculation.
† Agglutination.
‡ Traces of particulate matter were observed on repeat testing.
§ No agglutination.

from the low- and high-dose groups and 2 seals from the control group were killed at 5 and 10 wk postinoculation (PI) (Table I).

Two groups of 6 mice each were inoculated by stomach intubation with 10 *T. gondii* oocysts. Another group of 6 mice was used as uninoculated controls. One group of inoculated mice was killed at 5 wk PI, and the other inoculated and negative control groups were killed at 10 wk PI. Blood samples from each group were collected from the heart immediately after death.

Approximately 15 g of brain tissue (seal nos. 102 and 104) or muscle (seal nos. 101 and 103) removed postmortem from each of the 4 *T. gondii*–inoculated seals was fed to 4 cats (nos. 7, 11, and 21, 15, respectively). One cat was maintained as an uninoculated control. Blood samples from each cat were collected preinoculation and approximately 19 or 23 wk PI. Feces passed by each cat were collected at least twice before inoculation and then daily from inoculation to 5–7 wk PI.

Necropsies

Mice were killed by cervical dislocation. The brain of each mouse was removed and fixed for at least 1 wk in 10% formalin before further processing. Seals were sedated with Diazepam (Sabex Inc., Boucherville, Quebec, Canada) (5 mg per 50 kg body weight) and killed with a captive bolt gun, followed by exsanguination. The skin and blubber were removed from each seal and the entire carcass examined for signs of abnormality. Samples were collected from the following organs and tissues: liver, lungs, spleen, kidneys, brain, eyes, distal ileum, lymph nodes, and muscle from the front and rear flippers, pectoral, longissimus dorsi, psoas minor, diaphragm, tongue, and heart. A portion of each sample was fixed in 10% formalin for histology. The remainder of the muscle samples and brains were kept at 4 C and transported to the CFIA's Centre for Animal Parasitology, Saskatoon, Saskatchewan, for infectivity studies. Fecal samples were collected from the rectum of each seal for parasitological examination.

Assays

Histology: Formalin-fixed tissues from all seals and mice were processed by standard histological techniques, sectioned, 5 μm thick, and stained with hematoxylin and eosin (HE). For immunohistochemistry, 4-μm-thick sections were cut from paraffin-embedded blocks of formalin-fixed tissues and stained with an anti–*T. gondii* antibody raised in rabbits (Lindsay and Dubey, 1989). Immunostained sections prepared from 78 tissue blocks from the 4 inoculated seals were examined for *T. gondii*. All HE and immunostained sections were examined using a compound microscope and data recorded with a digital camera.

Serology: Sera from seals, mice, and cats were tested for the presence of anti–*T. gondii* immunoglobulin G antibodies using a modified agglutination test (MAT) kit, as per manufacturer's instructions (Bio-Mérieux, Lyon, France). Serum samples were diluted and tested at 1:40 and 1: 4,000, and sera from 2 cats experimentally infected with *T. gondii* at the Animal Parasite Diseases Laboratory of the United States Department of Agriculture, Beltsville, Maryland, were used as positive controls.

Fecal examination: Fecal samples collected from cats and seals were processed by standard sucrose flotation and examined for the presence of oocysts (Gajadhar, 1994). Briefly, approximately 5 g of feces were suspended in Sheather's solution (specific gravity 1.26), filtered through 2 layers of cheesecloth, and centrifuged at 400 *g* for 10 min. A cover slip that was placed on top of the filled centrifuge tube was removed and examined under a compound microscope at ×100 and ×400 magnifications.

RESULTS

The 4 seals inoculated with *T. gondii* oocysts occasionally exhibited increased activity, anxiety, and aggression, starting just before 5 wk PI. Such behavioral changes were not observed in control seals. Postmortem examination did not reveal any gross lesions or abnormalities other than a lung abscess in seal no. 104, which had been inoculated with 10,000 oocysts and killed at 5 wk PI. A bacteriological etiology of the abscess was confirmed by histology. Flotation tests performed on fecal samples obtained from seals did not reveal any oocysts. Results of the serology indicated that sera from all 4 seals inoculated with *T. gondii* were positive at both 1:40 and 1:4,000 dilutions (Table I). Sera collected from all seals before inoculation were negative, but some later samples from the negative control seals had particulate matter in the MAT.

Careful examination of the more than 500 HE–stained sections of sampled tissues collected from the infected and uninfected seals did not reveal any evidence of *T. gondii*. Similarly, evidence of *T. gondii* was not found in any of the immunohistochemically stained sections of 3 inoculated seals (nos. 101, 103, and 104) or of the control seals. A single anti–*T. gondii*-stained tissue cystlike structure was present in a brain section of seal no. 102 (Fig. 1).

Serum samples collected from all 4 cats before inoculation were negative on the MAT, whereas subsequent serum collections were positive. Fecal flotations performed on fecal samples from these cats were negative for oocysts at preinoculation and for 5 to 12 days PI (Fig. 2). Shedding of oocysts in all 4 cats lasted for about 1 week, and except for 1 case, only a few oocysts per gram of feces were shed. The control cat remained negative both serologically and on fecal examination.

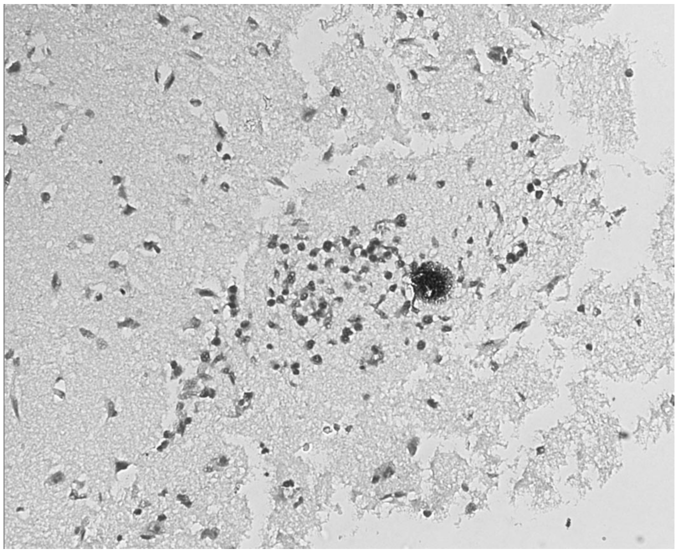

FIGURE 1. Histologic section of seal brain (no. 102) containing a *Toxoplasma gondii* cyst, which reacted to an immunohistochemical stain.

Most positive control mice and those infected with *T. gondii* for infectivity and virulence evaluation of the parasite became ill or died 7–14 days PI. Sera collected at 5 and 10 wk PI from positive control mice were MAT positive at 1:40 and 1:4,000 dilutions, and histology and immunohistochemistry of brain sections revealed *T. gondii* cysts. No clinical sign was observed in the negative control mice, and they were serologically and histologically negative for *T. gondii*.

DISCUSSION

This study describes the first experimental infection of *T. gondii* in a marine mammal. This study demonstrates that oocysts that are shed by cats and are allowed to sporulate are capable of establishing infections in seals. It shows that seals can serve as suitable intermediate hosts for *T. gondii* and might play a role in the establishment and transmission of the parasite in the marine environment. Evidence of the viability of *T. gondii* in marine mammals was previously demonstrated when live parasites were isolated from tissues of naturally infected sea otters (Cole et al., 2000; Lindsay, Thomas et al., 2001; Miller et al., 2001). All other findings of toxoplasmosis in marine mammals were by histological or immunological techniques and do not indicate the viability of the infection.

Although this study demonstrated the susceptibility of seals to sporulated oocysts, the source of natural *T. gondii* infection in marine mammals is unknown. Two possible sources are oocysts shed by domestic, feral, or wild felids and carried in freshwater runoffs and oocysts dumped with domestic cat litter into sewer systems, which empty into fresh water and coastal marine waters. Unsporulated oocysts entering the marine environment may not be infectious because the cool temperatures and salinity might inhibit sporulation. Although a recent study (Lindsay, Phelps et al., 2001) has shown that sporulated *T. gondii* oocysts remain viable after brief periods in saltwater, the effect of pro-

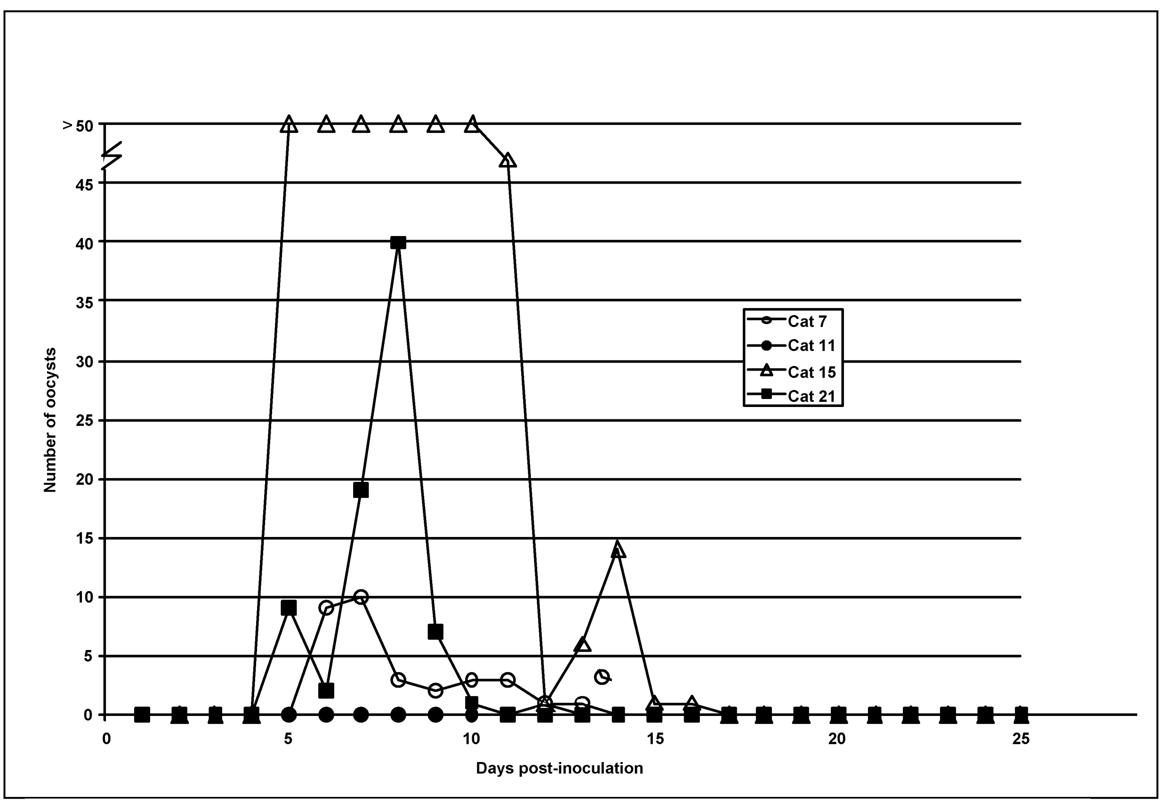

FIGURE 2. Shedding of oocysts (per 5 g of feces) by 4 cats that were fed brain or muscle from 4 seals experimentally inoculated with *Toxoplasma gondii*.

longed exposure to saltwater on the viability of sporulated oocysts is unknown. The dilution in marine environments reduces the chance of direct ingestion of oocysts; however, there is evidence to support the concentration and transmission of coccidian oocysts by filter-feeding benthic invertebrates (Graczyk et al., 1999; Lindsay, Phelps et al., 2001). Because benthic invertebrates are a food source for some marine mammals, such as sea otters and walruses, oocyst ingestion might be responsible for initiating reservoirs or cycles of *T. gondii* in the marine habitat.

The results of this study indicate that there may be major differences in susceptibility between marine mammals and terrestrial vertebrates to *T. gondii*. Whereas control mice became ill or died from few oocysts, few clinical signs were observed in seals infected with 100 or 10,000 oocysts. Also, tissue cysts were readily found in the brains of inoculated mice but were rare in the seals, being found in 1 animal only. These differences may be due to the variation in host susceptibility to the VEG strain of *T. gondii*. It is also possible that the pathology observed in natural cases of toxoplasmosis in seals and other marine mammals has been due to similarly low dose levels but more virulent strains of the parasite. This may indicate that marine host species do not readily adapt to *T. gondii* strains, which originate from terrestrial environments. Perhaps, if we had used a higher dose of oocysts or another strain of *T. gondii*, high morbidity and mortality might have resulted in the seals, corresponding with several reports of natural toxoplasmosis in marine mammals. However, it is unlikely that marine animals would be exposed naturally to high numbers of oocysts. Thus, the doses used in this study were reasonable, and the lack of severe disease was probably because of factors such as strain and host susceptibility.

From current knowledge on the biology of *T. gondii*, it appears that transmission from infected seals and other marine mammals could occur by predation or transplacentally to offspring. A case of transplacental toxoplasmosis in dolphins was recently described (Resendes et al., 2002). Successful transplacental transmission in marine mammals would help maintain parasite viability and establish the organism in the marine environment. There is no evidence to suggest that the duration of viability of *T. gondii* in marine intermediate hosts would be different from the prolonged viability in terrestrial hosts. An additional mode of transmission in the marine environment could be through paratenic invertebrate hosts, which feed on tissues of decaying carcasses. The cold ambient temperatures in the arctic and temperate environments would contribute to the extended viability of tissue cysts outside of their normal homeotherm host.

The finding of only 1 *T. gondii* tissue cyst in the 4 infected seals is consistent with the establishment of low-level infections and may be similar to naturally occurring infections. The reason why a tissue cyst was observed only in a low-dose animal, and not in any of the high-dose animals, is not known but is probably related to the low numbers of experimental animals and the relative insensitivity of current diagnostic methods in detecting *T. gondii* tissue cysts. Samples collected from the many different organs and tissues in the carcasses were examined by methods known to detect tissue cysts, and parasites were detected in sections of brains of positive control mice. Even though tissue cysts were not found in 3 of the seals, all animals were shown to be positive by bioassay using cats and by serology. Bioassay using cats is very sensitive (Dubey, 2001), can process much larger amounts of tissues than can be tested by stained sections, and the results can be used to assess food safety risks. The results of this study show that negative histological (HE stain and immunohistochemistry) results cannot be relied on for ruling out *T. gondii* as a cause of disease or to declare tissues safe for consumption. The bioassay results indicate that humans who consume raw or inadequately prepared seal meat could be at risk of acquiring toxoplasmosis; this is supported by recent cases among the Inuit population (L. Measures, pers. comm.). Further studies are underway to assess the infectivity of *T. gondii* in seal meat present in country foods.

The serological results of this study were consistent with expectations based on the experimental design. However, results should be interpreted with caution because the MAT test used has not been validated in marine mammals and the number of animals used in this study was too small to accurately estimate test characteristics such as sensitivity and specificity. Two of the negative control seals had agglutination-like particles in their serum when tested from week 3 to week 10 PI and could be misread as a positive reaction. It could not be determined whether these were antibody-mediated nonspecific reactions or physical phenomena due to other factors that mimicked agglutination. Test validation data based on a larger number of animals with known disease status are required to improve the interpretation of serological assay results from marine mammals.

The significance of behavioral changes in seals inoculated with *T. gondii* is not known. Seals used in this study were maintained in an environment that limited stress and exposure to pollution and pathogens. The absence of these factors might have increased the tolerance of seals to the parasites in this study. Environmental stressors can play a role in the susceptibility of animals to disease. Chemical pollution in the environment, in combination with exposure to multiple pathogens, probably has an adverse effect on animal health and survival. Fecal pollution can introduce pathogens such as *T. gondii*, *Cryptosporidium parvum*, *Sarcocystis neurona*, *Giardia lamblia*, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp., and *Vibrio* sp. Indeed, fatal protozoan encephalitis due to *T. gondii* in association with other pathogens in marine mammals has been reported (Mikaelian et al., 2000; Lindsay, Thomas et al., 2001; Miller et al., 2001). Such concurrent infections may be necessary for some strains of *T. gondii* or other pathogens to cause serious disease. The effect of individual pathogens in experiments on animals may not be an accurate measure of natural infections, and caution should be exercised when drawing conclusions on *T. gondii* infection in seals or other marine mammals based solely on the results of this study. This study has provided essential information concerning the susceptibility of seals to *T. gondii* oocysts. Further carefully designed experiments using other strains of *T. gondii*, additional doses, and other host species are necessary to determine the source and direct effect of toxoplasmosis in marine mammals.

ACKNOWLEDGMENTS

The authors gratefully acknowledge France Boily, Valérie Begin, Manón Cote, Magali Houde, Caroline Levesque, and Manón Simard at the Maurice-Lamontagne Institute, Fisheries and Oceans Canada, Mont-

Joli, Quebec, Canada, for assistance in handling and caring of seals, necropsies, and sample collection. Thanks also to Brad Scandrett, Mike Clancy, Shaun Dergousoff, Terry Steeves-Gurnsey, and Erin Zachar at the Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada, for support in animal care, serology, fecal analysis, and photography. Immunohistochemistry assays were performed by Keith West and staff at the Prairie Diagnostic Services, Saskatoon, Saskatchewan, Canada.

LITERATURE CITED

ARAMINI, J. J., C. STEPHEN, J. P. DUBEY, C. ENGELSTOFT, H. SCHWANTJE, AND C. RIBBLE. 1999. Potential contamination of drinking water with *Toxoplasma gondii* oocysts. Epidemiology and Infection **122:** 305–315.

BUERGELT, C. D., AND R. K. BONDE. 1983. Toxoplasmic meningoencephalitis in a West Indian manatee. Journal of American Veterinary Medical Association **183:** 1294–1296.

COLE, R. A., D. S. LINDSAY, D. K. HOWE, C. L. RODERICK, J. P. DUBEY, N. J. THOMAS, AND L. A. BAETEN. 2000. Biological and molecular characterizations of *Toxoplasma gondii* strains obtained from southern sea otters (*Enhydra lutris nereis*). Journal of Parasitology **86:** 526–530.

CRUICKSHANK, J. J., D. M. HAINES, N. C. PALMER, AND D. J. ST. AUBIN. 1990. Cysts of a *Toxoplasma*-like organism in an Atlantic bottlenose dolphin. Canadian Veterinary Journal **31:** 213–215.

DUBEY, J. P. 2001. Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. Journal of Parasitology **87:** 215–219.

———, J. K. LUNNEY, S. K. SHEN, D. C. H. KWOK, D. A. ASHFORD, AND P. THUILEZ. 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. Journal of Parasitology **82:** 438–443.

GAJADHAR, A. A. 1994. Host specificity studies and oocyst description of *Cryptosporidium* sp. isolated from ostriches. Parasitology Research **80:** 316–319.

GRACZYK, T. K., R. FAYER, E. J. LEWIS, J. M. TROUT, AND C. A. FARLEY. 1999. *Cryptosporidium* oocysts in bent mussels (*Ischadium recurvum*) in the Chesapeake Bay. Parasitology Research **85:** 518–521.

LAMBOURN, D. M., S. J. JEFFRIES, AND J. P. DUBEY. 2001. Seroprevalence of *Toxoplasma gondii* in harbor seals (*Phoca vitulina*) in Southern Puget Sound, Washington. Journal of Parasitology **87:** 1196–1197.

LINDSAY, D. S., AND J. P. DUBEY. 1989. Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. American Journal of Veterinary Research **50:** 1981–1983.

———, K. K. PHELPS, S. A. SMITH, G. FLICK, S. S. SUMNER, AND J. P. DUBEY. 2001. Removal of *Toxoplasma gondii* oocysts from sea water by eastern oysters (*Crassostrea virginica*). Journal of Eukaryotic Microbiology **48:** 197S–198S.

———, N. J. THOMAS, A. C. ROSYPAL, AND J. P. DUBEY. 2001. Dual *Sarcocystis neurona* and *Toxoplasma gondii* infection in a Northern sea otter from Washington State, USA. Veterinary Parasitology **97:** 319–327.

MIGAKI, G., J. F. ALLEN, AND H. W. CASEY. 1977. Toxoplasmosis in a California sea lion (*Zalophus californianus*). American Journal of Veterinary Research **38:** 135–136.

MIKAELIAN, I., J. BOISCLAIR, J. P. DUBEY, S. KENNEDY, AND D. MARTINEAU. 2000. Toxoplasmosis in Beluga Whales (*Delphinapterus leucas*) from the St Lawrence Estuary: Two case reports and a serological survey. Journal of Comparative Pathology **122:** 73–76.

MILLER, M. A., I. A. GARDNER, C. KREUDER, D. M. PARADIES, K. R. WORCESTER, D. A. JESSUP, E. DODD, M. D. HARRIS, J. A. AMES, A. E. PACKHAM, AND P. A. CONRAD. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology **32:** 997–1006.

———, K. SVERLOW, P. R. CROSBIE, B. C. BARR, L. J. LOWENSTINE, F. M. GULLAND, A. PACKHAM, AND P. A. CONRAD. 2001. Isolation and characterization of two parasitic protozoa from Pacific harbor seal (*Phoca vitulina richardsi*) with meningoencephalomyelitis. Journal of Parasitology **87:** 816–822.

RESENDES, A. R., S. ALMERIA, J. P. DUBEY, E. OBÓN, C. JUAN-SALLÉS, E. DEGOLLADA, F. ALEGRE, O. CABEZÓN, S. PONT, AND M. DOMINGO. 2002. Disseminated toxoplasmosis in a Mediterranean pregnant Risso's dolphin (*Grampus griseus*) with transplacental fetal infection. Journal of Parasitology **88:** 1029–1032.

TENTER, A. M., A. R. HECKEROTH, AND L. M. WEISS. 2000. *Toxoplasma gondii*: From animals to humans. International Journal for Parasitology **30:** 1217–1258.

WALLACE, G. D. 1972. Experimental transmission of *Toxoplasma gondii* by cockroaches. Journal of Infectious Diseases **126:** 545.